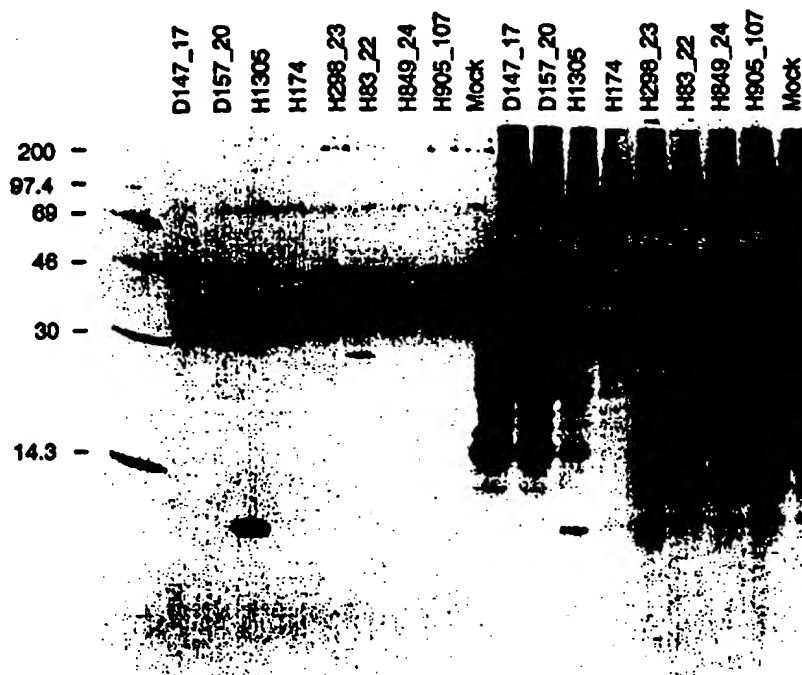




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/12, 15/19, C07K 14/52, 14/47, A61K 38/17, 38/19, C12N 5/10</b>		<b>A2</b>	(11) International Publication Number: <b>WO 97/46683</b>
			(43) International Publication Date: 11 December 1997 (11.12.97)
(21) International Application Number: <b>PCT/US97/09878</b>		(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: <b>6 June 1997 (06.06.97)</b>			
(30) Priority Data: <b>08/659,224 7 June 1996 (07.06.96) US</b>		<b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>	
(71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US).			
(72) Inventors: JACOBS, Kenneth; 151 Beaumont Avenue, Newton, MA 02160 (US). MCCOY, John, M.; 56 Howard Street, Reading, MA 01867 (US). LAVALLIE, Edward, R.; 90 Green Meadow Drive, Tewksbury, MA 01876 (US). RACIE, Lisa, A.; 124 School Street, Acton, MA 01720 (US). MERBERG, David; 2 Orchard Drive, Acton, MA 01720 (US). TREACY, Maurice; 93 Walcott Road, Chestnut Hill, MA 02167 (US). EVANS, Cheryl; Apartment #21, 35 Bellvista Road, Brookline, MA 02146 (US). BOWMAN, Michael; 50 Aldrich Road, Canton, MA 02021 (US). SPAULDING, Vikki; 11 Meadowbank Road, Billerica, MA 01821 (US).			
(74) Agent: SPRUNGER, Suzanne, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).			

(54) Title: POLYNUCLEOTIDE ENCODING SECRETED PROTEINS



## (57) Abstract

The invention provides 13 clones "AZ302-1" isolated from human colon; "AU139-2", "AU105-14", and "AJ147-1" from human adult testes; "AS268-1", "AS264-3", "AS301-2", "AS162-1" AND "AS86-1" from human fetal brain; "D147-17" from human PBMC; "075-9" from human dendritic cells; "AM262-11" from human fetal kidney and clone "AR28-1" from human adult retina comprising polynucleotides encoding secreted proteins, using methods selective for cDNAs encoding secreted proteins.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## POLYNUCLEOTIDE ENCODING SECRETED PROTEINS

5

FIELD OF THE INVENTION

The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins.

10

BACKGROUND OF THE INVENTION

Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered protein (i.e., partial DNA/amino acid sequence of the protein in the case of hybridization cloning; activity of the protein in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, have advanced the state of the art by making available large numbers of DNA/amino acid sequences for proteins that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, or by virtue of the cell or tissue source in the case of PCR-based techniques. It is to these proteins and the polynucleotides encoding them that the present invention is directed.

25

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 351 to nucleotide 506;
- (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AZ302\_1 deposited under accession number ATCC 98076;
- (d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AZ302\_1 deposited under accession number ATCC 98076;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AZ302\_1 deposited under accession number ATCC 98076;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AZ302\_1 deposited under accession number ATCC 98076;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:3;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:3 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(d) above;

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:2 from nucleotide 351 to nucleotide 506; the nucleotide sequence of the full length protein coding sequence of clone AZ302\_1 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AZ302\_1 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AZ302\_1 deposited under accession number ATCC 98076.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:2, SEQ ID NO:1 or SEQ ID NO:4.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:3;
- (b) fragments of the amino acid sequence of SEQ ID NO:3; and
- (c) the amino acid sequence encoded by the cDNA insert of clone AZ302\_1 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:3.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 23 to nucleotide 517;

(c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AU139\_2 deposited under accession number ATCC 98076;

(d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AU139\_2 deposited under accession number ATCC 98076;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AU139\_2 deposited under accession number ATCC 98076;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AU139\_2 deposited under accession number ATCC 98076;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(d) above;

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:5 from nucleotide 23 to nucleotide 517; the nucleotide sequence of the full length protein coding sequence of clone AU139\_2 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AU139\_2 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AU139\_2 deposited under accession number ATCC 98076. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6 from amino acid 35 to amino acid 115.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:5 or SEQ ID NO:7.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:6;

(b) the amino acid sequence of SEQ ID NO:6 from amino acid 35 to amino acid 115;

(c) fragments of the amino acid sequence of SEQ ID NO:6; and

(d) the amino acid sequence encoded by the cDNA insert of clone

5 AU139\_2 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:6 or the amino acid sequence of SEQ ID NO:6 from amino acid 35 to amino acid 115.

In one embodiment, the present invention provides a composition comprising an  
10 isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 288 to nucleotide 629;

15 (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 441 to nucleotide 629;

(d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AU105\_14 deposited under accession number ATCC 98076;

20 (e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AU105\_14 deposited under accession number ATCC 98076;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AU105\_14 deposited under accession number ATCC 98076;

25 (g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AU105\_14 deposited under accession number ATCC 98076;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:9;

(i) a polynucleotide encoding a protein comprising a fragment of the  
30 amino acid sequence of SEQ ID NO:9 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above; and

(l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:8 from nucleotide 288 to nucleotide 629; the nucleotide sequence of SEQ ID NO:8 from nucleotide 441 to nucleotide 629; the nucleotide sequence of the full length protein coding sequence of clone AU105\_14 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AU105\_14 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AU105\_14 deposited under accession number ATCC 98076. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:9 from amino acid 25 to amino acid 44.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:8 or SEQ ID NO:10.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:9;
  - (b) the amino acid sequence of SEQ ID NO:9 from amino acid 25 to amino acid 44;
  - (c) fragments of the amino acid sequence of SEQ ID NO:9; and
  - (d) the amino acid sequence encoded by the cDNA insert of clone AU105\_14 deposited under accession number ATCC 98076;
- the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:9 or the amino acid sequence of SEQ ID NO:9 from amino acid 25 to amino acid 44.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 164 to nucleotide 298;
- (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS268\_1 deposited under accession number ATCC 98076;

(d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AS268\_1 deposited under accession number ATCC 98076;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS268\_1 deposited under accession number ATCC 98076;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS268\_1 deposited under accession number ATCC 98076;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(d) above;

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:11 from nucleotide 164 to nucleotide 298; the nucleotide sequence of the full length protein coding sequence of clone AS268\_1 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AS268\_1 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AS268\_1 deposited under accession number ATCC 98076.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:11 or SEQ ID NO:13.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:12;

(b) fragments of the amino acid sequence of SEQ ID NO:12; and

(c) the amino acid sequence encoded by the cDNA insert of clone AS268\_1 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:12.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:



- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 from nucleotide 254 to nucleotide 681;
- 5 (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone D147\_17 deposited under accession number ATCC 98076;
- (d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone D147\_17 deposited under accession number ATCC 98076;
- 10 (e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone D147\_17 deposited under accession number ATCC 98076;
- (f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone D147\_17 deposited under accession number ATCC 98076;
- 15 (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:16;
- (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:16 having biological activity;
- (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-
- 20 (d) above;
- (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above and
- (k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h).
- 25 Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:15 from nucleotide 254 to nucleotide 681; the nucleotide sequence of the full length protein coding sequence of clone D147\_17 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone D147\_17 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes
- 30 the full length or mature protein encoded by the cDNA insert of clone D147\_17 deposited under accession number ATCC 98076. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:16 from amino acid 73 to amino acid 129.
- Other embodiments provide the gene corresponding to the cDNA sequence of SEQ
- 35 ID NO:15, SEQ ID NO:14 or SEQ ID NO:17.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:16;
- 5 (b) the amino acid sequence of SEQ ID NO:16 from amino acid 73 to amino acid 129;
- (c) fragments of the amino acid sequence of SEQ ID NO:16; and
- (d) the amino acid sequence encoded by the cDNA insert of clone D147\_17 deposited under accession number ATCC 98076;
- 10 the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:16 or the amino acid sequence of SEQ ID NO:16 from amino acid 73 to amino acid 129.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 15 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:18;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:18 from nucleotide 28 to nucleotide 388;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:18 from nucleotide 76 to nucleotide 388;
- 20 (d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone O75\_9 deposited under accession number ATCC 98076;
- (e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone O75\_9 deposited under accession number ATCC 98076;
- 25 (f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone O75\_9 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone O75\_9 deposited under accession number ATCC 98076;
- 30 (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:19;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:19 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above; and

5 (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:18 from nucleotide 28 to nucleotide 388; the nucleotide sequence of SEQ ID NO:18 from nucleotide 76 to nucleotide 388; the nucleotide sequence of the full length protein coding  
10 sequence of clone O75\_9 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone O75\_9 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone O75\_9 deposited under accession number ATCC 98076.

15 Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:18 or SEQ ID NO:20.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

20 (a) the amino acid sequence of SEQ ID NO:19;  
(b) fragments of the amino acid sequence of SEQ ID NO:19; and  
(c) the amino acid sequence encoded by the cDNA insert of clone O75\_9 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins. Preferably such protein  
25 comprises the amino acid sequence of SEQ ID NO:19.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:21;  
30 (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:21 from nucleotide 75 to nucleotide 419;  
(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:21 from nucleotide 132 to nucleotide 419;

(d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AJ147\_1 deposited under accession number ATCC 98076;

(e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AJ147\_1 deposited under accession number ATCC 98076;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AJ147\_1 deposited under accession number ATCC 98076;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AJ147\_1 deposited under accession number ATCC 98076;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:22;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:22 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:21 from nucleotide 75 to nucleotide 419; the nucleotide sequence of SEQ ID NO:21 from nucleotide 132 to nucleotide 419; the nucleotide sequence of the full length protein coding sequence of clone AJ147\_1 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AJ147\_1 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AJ147\_1 deposited under accession number ATCC 98076.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:21 or SEQ ID NO:23.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:22;

(b) fragments of the amino acid sequence of SEQ ID NO:22; and

(c) the amino acid sequence encoded by the cDNA insert of clone AJ147\_1 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:22.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 5 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:24;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:24 from nucleotide 69 to nucleotide 377;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID  
10 NO:24 from nucleotide 120 to nucleotide 377;
- (d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AM262\_11 deposited under accession number ATCC 98076;
- (e) a polynucleotide encoding the full length protein encoded by the  
15 cDNA insert of clone AM262\_11 deposited under accession number ATCC 98076;
- (f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AM262\_11 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding the mature protein encoded by the cDNA  
20 insert of clone AM262\_11 deposited under accession number ATCC 98076;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:25;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:25 having biological activity;
- 25 (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:24  
30 from nucleotide 69 to nucleotide 377; the nucleotide sequence of SEQ ID NO:24 from nucleotide 120 to nucleotide 377; the nucleotide sequence of the full length protein coding sequence of clone AM262\_11 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AM262\_11 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide  
35 encodes the full length or mature protein encoded by the cDNA insert of clone AM262\_11

deposited under accession number ATCC 98076. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:25 from amino acid 14 to amino acid 81.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:24.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:25;
- (b) the amino acid sequence of SEQ ID NO:25 from amino acid 14 to amino acid 81;
- (c) fragments of the amino acid sequence of SEQ ID NO:25; and
- (d) the amino acid sequence encoded by the cDNA insert of clone AM262\_11 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:25 or the amino acid sequence of SEQ ID NO:25 from amino acid 14 to amino acid 81.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:26;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:26 from nucleotide 110 to nucleotide 448;
- (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AR28\_1 deposited under accession number ATCC 98076;
- (d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AR28\_1 deposited under accession number ATCC 98076;
- (e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR28\_1 deposited under accession number ATCC 98076;
- (f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR28\_1 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:27;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:27 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(d) above;

5 (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:26  
10 from nucleotide 110 to nucleotide 448; the nucleotide sequence of the full length protein coding sequence of clone AR28\_1 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AR28\_1 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AR28\_1 deposited  
15 under accession number ATCC 98076. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:27 from amino acid 15 to amino acid 78.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:26 or SEQ ID NO:28.

20 In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:27;  
(b) the amino acid sequence of SEQ ID NO:27 from amino acid 15 to  
25 amino acid 78;

(c) fragments of the amino acid sequence of SEQ ID NO:27; and

(d) the amino acid sequence encoded by the cDNA insert of clone AR28\_1 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins. Preferably such protein  
30 comprises the amino acid sequence of SEQ ID NO:27 or the amino acid sequence of SEQ ID NO:27 from amino acid 15 to amino acid 78.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID  
35 NO:30;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:30 from nucleotide 230 to nucleotide 541;

(c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS162\_1 deposited under accession number ATCC 98076;

(d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AS162\_1 deposited under accession number ATCC 98076;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS162\_1 deposited under accession number ATCC 98076;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS162\_1 deposited under accession number ATCC 98076;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:31;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:31 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(d) above;

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:30 from nucleotide 230 to nucleotide 541; the nucleotide sequence of the full length protein coding sequence of clone AS162\_1 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AS162\_1 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AS162\_1 deposited under accession number ATCC 98076. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:31 from amino acid 5 to amino acid 25.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:30, SEQ ID NO:29 or SEQ ID NO:32.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:31;



(b) the amino acid sequence of SEQ ID NO:31 from amino acid 5 to amino acid 25;

(c) fragments of the amino acid sequence of SEQ ID NO:31; and

(d) the amino acid sequence encoded by the cDNA insert of clone

5 AS162\_1 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:31 or the amino acid sequence of SEQ ID NO:31 from amino acid 5 to amino acid 25.

In one embodiment, the present invention provides a composition comprising an  
10 isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:34;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:34 from nucleotide 202 to nucleotide 467;

15 (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:34 from nucleotide 241 to nucleotide 467;

(d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS264\_3 deposited under accession number ATCC 98076;

20 (e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AS264\_3 deposited under accession number ATCC 98076;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS264\_3 deposited under accession number ATCC 98076;

25 (g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS264\_3 deposited under accession number ATCC 98076;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:35;

30 (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:35 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:34 from nucleotide 202 to nucleotide 467; the nucleotide sequence of SEQ ID NO:34 from nucleotide 241 to nucleotide 467; the nucleotide sequence of the full length protein coding sequence of clone AS264\_3 deposited under accession number ATCC 98076; or the nucleotide  
5 sequence of the mature protein coding sequence of clone AS264\_3 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AS264\_3 deposited under accession number ATCC 98076.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ  
10 ID NO:34, SEQ ID NO:33 or SEQ ID NO:36.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:35;
- 15 (b) fragments of the amino acid sequence of SEQ ID NO:35; and
- (c) the amino acid sequence encoded by the cDNA insert of clone AS264\_3 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:35.

20 In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:38;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID  
25 NO:38 from nucleotide 173 to nucleotide 579;
- (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS301\_2 deposited under accession number ATCC 98076;
- (d) a polynucleotide encoding the full length protein encoded by the  
30 cDNA insert of clone AS301\_2 deposited under accession number ATCC 98076;
- (e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS301\_2 deposited under accession number ATCC 98076;
- (f) a polynucleotide encoding the mature protein encoded by the cDNA  
35 insert of clone AS301\_2 deposited under accession number ATCC 98076;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:39;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:39 having biological activity;

5 (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(d) above;

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:38  
10 from nucleotide 173 to nucleotide 579; the nucleotide sequence of the full length protein coding sequence of clone AS301\_2 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AS301\_2 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AS301\_2 deposited  
15 under accession number ATCC 98076.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:38, SEQ ID NO:37 or SEQ ID NO:40.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group  
20 consisting of:

- (a) the amino acid sequence of SEQ ID NO:39;
- (b) fragments of the amino acid sequence of SEQ ID NO:39; and
- (c) the amino acid sequence encoded by the cDNA insert of clone AS301\_2 deposited under accession number ATCC 98076;

25 the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:39.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID  
30 NO:42;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:42 from nucleotide 363 to nucleotide 593;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:42 from nucleotide 483 to nucleotide 593;

(d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS86\_1 deposited under accession number ATCC 98076;

(e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AS86\_1 deposited under accession number ATCC 98076;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS86\_1 deposited under accession number ATCC 98076;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS86\_1 deposited under accession number ATCC 98076;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:43;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:43 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above; and

(l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:42 from nucleotide 363 to nucleotide 593; the nucleotide sequence of SEQ ID NO:42 from nucleotide 483 to nucleotide 593; the nucleotide sequence of the full length protein coding sequence of clone AS86\_1 deposited under accession number ATCC 98076; or the nucleotide sequence of the mature protein coding sequence of clone AS86\_1 deposited under accession number ATCC 98076. In other preferred embodiments, the polynucleotide encodes the full length or mature protein encoded by the cDNA insert of clone AS86\_1 deposited under accession number ATCC 98076.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:42, SEQ ID NO:41 or SEQ ID NO:44.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:43;

(b) fragments of the amino acid sequence of SEQ ID NO:43; and

(c) the amino acid sequence encoded by the cDNA insert of clone AS86\_I deposited under accession number ATCC 98076; the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:43.

5 In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with such polynucleotide compositions.

Processes are also provided for producing a protein, which comprise:

- 10 (a) growing a culture of the host cell transformed with such polynucleotide compositions in a suitable culture medium; and  
(b) purifying the protein from the culture.

The protein produced according to such methods is also provided by the present invention. Preferred embodiments include those in which the protein produced by such process is a mature form of the protein.

15 Protein compositions of the present invention may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody which specifically reacts with such protein are also provided by the present invention.

Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of  
20 a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

#### BRIEF DESCRIPTION OF FIGURES

- Fig. 1 is an autoradiograph demonstrating the expression of D147\_17 in COS cells.  
25 Fig. 2 is an autoradiograph demonstrating the expression of AM262\_11 in COS cells.

#### DETAILED DESCRIPTION

##### ISOLATED PROTEINS AND POLYNUCLEOTIDES

30 Nucleotide and amino acid sequences are reported below for each clone and protein disclosed in the present application. In some instances the sequences are preliminary and may include some incorrect or ambiguous bases or amino acids. The actual nucleotide sequence of each clone can readily be determined by sequencing of the deposited clone in accordance with known methods. The predicted amino acid sequence (both full length and mature) can then be determined from such nucleotide sequence. The amino acid sequence of the protein

encoded by a particular clone can also be determined by expression of the clone in a suitable host cell, collecting the protein and determining its sequence.

For each disclosed protein applicants have identified what they have determined to be the reading frame best identifiable with sequence information available at the time of filing.

5 Because of the partial ambiguity in reported sequence information, reported protein sequences include "Xaa" designators. These "Xaa" designators indicate either (1) a residue which cannot be identified because of nucleotide sequence ambiguity or (2) a stop codon in the determined nucleotide sequence where applicants believe one should not exist (if the nucleotide sequence were determined more accurately).

10 As used herein a "secreted" protein is one which, when expressed in a suitable host cell, is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g., receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins which are  
15 transported across the membrane of the endoplasmic reticulum.

#### Clone "AZ302\_1"

A polynucleotide of the present invention has been identified as clone "AZ302\_1". AZ302\_1 was isolated from a human colon (Caco-2 adenocarcinoma) cDNA library using  
20 methods which are selective for cDNAs encoding secreted proteins. AZ302\_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AZ302\_1 protein").

The nucleotide sequence of the 5' portion of AZ302\_1 as presently determined is reported in SEQ ID NO:1. An additional internal nucleotide sequence from AZ302\_1 as  
25 presently determined is reported in SEQ ID NO:2. What applicants believe is the proper reading frame and the predicted amino acid sequence encoded by such internal sequence is reported in SEQ ID NO:3. Additional nucleotide sequence from the 3' portion of AZ302\_1, including the polyA tail, is reported in SEQ ID NO:4.

The nucleotide sequence disclosed herein for AZ302\_1 was searched against the  
30 GenBank database using BLASTA/BLASTX and FASTA search protocols. AZ302\_1 demonstrated at least some homology with an EST identified as "ye83a03.r1 Homo sapiens cDNA clone 124300 5'" at accession number R02197 (BlastN). Based upon homology, AZ302\_1 proteins and each homologous protein or peptide may share at least some activity.

35 Clone "AU139\_2"

A polynucleotide of the present invention has been identified as clone "AU139\_2". AU139\_2 was isolated from a human adult testes cDNA library using methods which are selective for cDNAs encoding secreted proteins. AU139\_2 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AU139\_2 protein").

5        The nucleotide sequence of the 5' portion of AU139\_2 as presently determined is reported in SEQ ID NO:5. What applicants presently believe is the proper reading frame for the coding region is indicated in SEQ ID NO:6. The predicted acid sequence of the AU139\_2 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:6. Additional nucleotide sequence from the 3' portion of AU139\_2, including the polyA tail, is reported in SEQ ID NO:7.

10       The nucleotide sequence disclosed herein for AU139\_2 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. AU139\_2 demonstrated at least some homology with three ESTs identified as "EST16319 Homo sapiens cDNA 5' end" (accession number T30419, BlastN), "EST04080 Homo sapiens cDNA clone HFBDQ07" (accession number T06191, BlastN), and "EST108441 Rattus sp. cDNA 5'". Based upon homology, AU139\_2 proteins and each homologous protein or peptide may share at least some activity.

#### Clone "AU105\_14"

20       A polynucleotide of the present invention has been identified as clone "AU105\_14". AU105\_14 was isolated from a human adult testes cDNA library using methods which are selective for cDNAs encoding secreted proteins. AU105\_14 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AU105\_14 protein").

25       The nucleotide sequence of the 5' portion of AU105\_14 as presently determined is reported in SEQ ID NO:8. What applicants presently believe is the proper reading frame for the coding region is indicated in SEQ ID NO:9. The predicted acid sequence of the AU105\_14 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:9. Amino acids 1 to 51 are the predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 52. Additional nucleotide sequence from the 3' portion of AU105\_14, including the polyA tail, is reported in SEQ ID NO:10.

30       The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AU105\_14 should be approximately 2670 bp.

The nucleotide sequence disclosed herein for AU105\_14 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. No hits were found in the database.

5        Clone "AS268\_1"

A polynucleotide of the present invention has been identified as clone "AS268\_1". AS268\_1 was isolated from a human fetal brain cDNA library using methods which are selective for cDNAs encoding secreted proteins. AS268\_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AS268\_1 protein").

10        The nucleotide sequence of the 5' portion of AS268\_1 as presently determined is reported in SEQ ID NO:11. What applicants presently believe is the proper reading frame for the coding region is indicated in SEQ ID NO:12. The predicted acid sequence of the AS268\_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:12. Additional nucleotide sequence from the 3' portion of AS268\_1, including the polyA tail, is  
15        reported in SEQ ID NO:13.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AS268\_1 should be approximately 1800 bp.

The nucleotide sequence disclosed herein for AS268\_1 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. AS268\_1  
20        demonstrated at least some homology with the rabbit and murine ryanodine receptors (BlastN accession number M59743, BlastX accession number X83933). Ryanodine receptors have recently been shown to be the  $Ca^{2+}$  release channels of sarcoplasmic reticulum in both cardiac muscle and skeletal muscle. Based upon homology, AS268\_1 proteins and each homologous protein or peptide may share at least some activity.

25

Clone "D147\_17"

A polynucleotide of the present invention has been identified as clone "D147\_17". D147\_17 was isolated from a human PBMC cDNA library using methods which are selective for cDNAs encoding secreted proteins. D147\_17 is a full-length clone, including the entire  
30        coding sequence of a secreted protein (also referred to herein as "D147\_17 protein").

The nucleotide sequence of the 5' portion of D147\_17 as presently determined is reported in SEQ ID NO:14. An additional internal nucleotide sequence from D147\_17 as presently determined is reported in SEQ ID NO:15. What applicants believe is the proper reading frame and the predicted amino acid sequence encoded by such internal sequence is



reported in SEQ ID NO:16. Additional nucleotide sequence from the 3' portion of D147\_17, including the polyA tail, is reported in SEQ ID NO:17.

The nucleotide sequence disclosed herein for D147\_17 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. No hits were  
5 found in the database.

#### Clone "O75\_9"

A polynucleotide of the present invention has been identified as clone "O75\_9". O75\_9 was isolated from a human dendritic cells cDNA library using methods which are  
10 selective for cDNAs encoding secreted proteins. O75\_9 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "O75\_9 protein").

The nucleotide sequence of the 5' portion of O75\_9 as presently determined is reported in SEQ ID NO:18. What applicants presently believe is the proper reading frame for the coding region is indicated in SEQ ID NO:19. The predicted acid sequence of the O75\_9 protein  
15 corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:19. Amino acids 1 to 16 are the predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 17. Additional nucleotide sequence from the 3' portion of O75\_9, including the polyA tail, is reported in SEQ ID NO:20.

The nucleotide sequence disclosed herein for O75\_9 was searched against the  
20 GenBank database using BLASTA/BLASTX and FASTA search protocols. No hits were found in the database.

#### Clone "AJ147\_1"

A polynucleotide of the present invention has been identified as clone "AJ147\_1".  
25 AJ147\_1 was isolated from a human adult testes cDNA library using methods which are selective for cDNAs encoding secreted proteins. AJ147\_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AJ147\_1 protein").

The nucleotide sequence of the 5' portion of AJ147\_1 as presently determined is reported in SEQ ID NO:21. What applicants presently believe is the proper reading frame for  
30 the coding region is indicated in SEQ ID NO:22. The predicted acid sequence of the AJ147\_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:22. Amino acids 1 to 19 are the predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 20. Additional nucleotide sequence from the 3' portion of AJ147\_1, including the polyA tail, is reported in SEQ ID NO:23.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AJ147\_1 should be approximately 500 bp.

The nucleotide sequence disclosed herein for AJ147\_1 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. AJ147\_1 demonstrated at least some homology with murine calmegin (Meg 1)/calnexin (BlastN accession number D14117). Calmegin is a  $\text{Ca}^{2+}$ -binding protein that is specifically expressed in spermatogenesis. The highly regulated, specific and abundant expression of calmegin suggests that it plays an important role in spermatogenesis. Based upon homology, AJ147\_1 proteins and each homologous protein or peptide may share at least some activity.

10

Clone "AM262\_11"

A polynucleotide of the present invention has been identified as clone "AM262\_11". AM262\_11 was isolated from a human fetal kidney cDNA library using methods which are selective for cDNAs encoding secreted proteins. AM262\_11 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AM262\_11 protein").

The nucleotide sequence of AM262\_11 as presently determined is reported in SEQ ID NO:24. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the AM262\_11 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:25. Amino acids 1 to 17 are the predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 18.

The nucleotide sequence disclosed herein for AM262\_11 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. AM262\_11 demonstrated at least some identity with the human eotaxin precursor gene and protein (BlastN accession number U34780; this database entry was made subsequent to applicants' isolation of AM262\_11). Based upon homology, AM262\_11 proteins and each homologous protein or peptide may share at least some activity.

Clone "AR28\_1"

A polynucleotide of the present invention has been identified as clone "AR28\_1". AR28\_1 was isolated from a human adult retina cDNA library using methods which are selective for cDNAs encoding secreted proteins. AR28\_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AR28\_1 protein").

The nucleotide sequence of the 5' portion of AR28\_1 as presently determined is reported in SEQ ID NO:26. What applicants presently believe is the proper reading frame for

the coding region is indicated in SEQ ID NO:27. The predicted acid sequence of the AR28\_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:27. Additional nucleotide sequence from the 3' portion of AR28\_1, including the polyA tail, is reported in SEQ ID NO:28.

- 5           The nucleotide sequence disclosed herein for AR28\_1 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. No hits were found in the database.

Clone "AS162\_1"

- 10           A polynucleotide of the present invention has been identified as clone "AS162\_1". AS162\_1 was isolated from a human fetal brain cDNA library using methods which are selective for cDNAs encoding secreted proteins. AS162\_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AS162\_1 protein").

- 15           The nucleotide sequence of the 5' portion of AS162\_1 as presently determined is reported in SEQ ID NO:29. An additional internal nucleotide sequence from AS162\_1 as presently determined is reported in SEQ ID NO:30. What applicants believe is the proper reading frame and the predicted amino acid sequence encoded by such internal sequence is reported in SEQ ID NO:31. Additional nucleotide sequence from the 3' portion of AS162\_1, including the polyA tail, is reported in SEQ ID NO:32.

- 20           The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AS162\_1 should be approximately 1380 bp.

- The nucleotide sequence disclosed herein for AS162\_1 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. AS162\_1 demonstrated at least some identity with an EST identified as "ym96e05.s1 Homo sapiens cDNA clone 166784 3'" (accession number R88809, BlastN). Based upon identity, AS162\_1  
25           proteins and each identical protein or peptide may share at least some activity.

Clone "AS264\_3"

- A polynucleotide of the present invention has been identified as clone "AS264\_3".  
30           AS264\_3 was isolated from a human fetal brain cDNA library using methods which are selective for cDNAs encoding secreted proteins. AS264\_3 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AS264\_3 protein").

- The nucleotide sequence of the 5' portion of AS264\_3 as presently determined is reported in SEQ ID NO:33. An additional internal nucleotide sequence from AS264\_3 as  
35           presently determined is reported in SEQ ID NO:34. What applicants believe is the proper

reading frame and the predicted amino acid sequence encoded by such internal sequence is reported in SEQ ID NO:35. Amino acids 1 to 13 of SEQ ID NO:35 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 14. Additional nucleotide sequence from the 3' portion of AS264\_3, including the polyA tail, is reported in SEQ ID NO:36.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AS264\_3 should be approximately 3300 bp.

The nucleotide sequence disclosed herein for AS264\_3 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. AS264\_3 demonstrated at least some weak similarity to collagen. Based upon homology, AS264\_3 proteins and each homologous protein or peptide may share at least some activity.

#### Clone "AS301\_2"

A polynucleotide of the present invention has been identified as clone "AS301\_2". AS301\_2 was isolated from a human fetal brain cDNA library using methods which are selective for cDNAs encoding secreted proteins. AS301\_2 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AS301\_2 protein").

The nucleotide sequence of the 5' portion of AS301\_2 as presently determined is reported in SEQ ID NO:37. An additional internal nucleotide sequence from AS301\_2 as presently determined is reported in SEQ ID NO:38. What applicants believe is the proper reading frame and the predicted amino acid sequence encoded by such internal sequence is reported in SEQ ID NO:39. Additional nucleotide sequence from the 3' portion of AS301\_2, including the polyA tail, is reported in SEQ ID NO:40.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AS301\_2 should be approximately 2600 bp.

The nucleotide sequence disclosed herein for AS301\_2 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. AS301\_2 demonstrated at least some homology with ESTs identified as "yp82b08.r1 Homo sapiens cDNA clone 193911 5'" (BlastN accession number R83399), "ye66c02.r1 Homo sapiens cDNA clone 122690 5'", and "ym26e09.r1 Homo sapiens cDNA clone 49167 5'" (BlastN accession number H16691). Based upon homology, AS301\_2 proteins and each homologous protein or peptide may share at least some activity.

#### Clone "AS86\_1"

A polynucleotide of the present invention has been identified as clone "AS86\_1". AS86\_1 was isolated from a human fetal brain cDNA library using methods which are selective for cDNAs encoding secreted proteins. AS86\_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AS86\_1 protein").

5 The nucleotide sequence of the 5' portion of AS86\_1 as presently determined is reported in SEQ ID NO:41. An additional internal nucleotide sequence from AS86\_1 as presently determined is reported in SEQ ID NO:42. What applicants believe is the proper reading frame and the predicted amino acid sequence encoded by such internal sequence is reported in SEQ ID NO:43. Amino acids 1 to 40 of SEQ ID NO:43 are a predicted  
10 leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 41. Additional nucleotide sequence from the 3' portion of AS86\_1, including the polyA tail, is reported in SEQ ID NO:44.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AS86\_1 should be approximately 2122 bp.

15 The nucleotide sequence disclosed herein for AS86\_1 was searched against the GenBank database using BLASTA/BLASTX and FASTA search protocols. No hits were found in the database.

Figs. 1 and 2 are autoradiographs evidencing expression of clones of the present  
20 invention. All clones were expressed in COS cells.

#### Deposit of Clones

Clones AZ302\_1, AU139\_2, AU105\_14, AS268\_1, D147\_17, O75\_9, AJ147\_1, AM262\_11, AR28\_1, AS162\_1, AS264\_3, AS301\_2 and AS86\_1 were deposited on June 6,  
25 1996 with the American Type Culture Collection under accession number ATCC 98076, from which each clone comprising a particular polynucleotide is obtainable. Each clone has been transfected into separate bacterial cells (*E. coli*) in this composite deposit. Each clone can be removed from the vector in which it was deposited by performing an EcoRI/NotI digestion (5' cite, EcoRI; 3' cite, NotI) to produce the appropriately sized fragment for such clone  
30 (approximate clone size fragment are identified below). Bacterial cells containing a particular clone can be obtained from the composite deposit as follows:

An oligonucleotide probe or probes should be designed to the sequence that is known for that particular clone. This sequence can be derived from the sequences provided herein, or from a combination of those sequences. The sequence of the oligonucleotide probe that was

used to isolate each full-length clone is identified below, and should be most reliable in isolating the clone of interest.

	<u>Clone</u>	<u>Probe Sequence</u>
5	AZ302_1	SEQ ID NO:45
	AU139_2	SEQ ID NO:46
	AU105_14	SEQ ID NO:47
	AS268_1	SEQ ID NO:48
	DI47_17	SEQ ID NO:49
10	O75_9	SEQ ID NO:50
	AJ147_1	SEQ ID NO:51
	AM262_11	SEQ ID NO:52
	AR28_1	SEQ ID NO:53
	AS162_1	SEQ ID NO:54
15	AS264_3	SEQ ID NO:55
	AS301_2	SEQ ID NO:56
	AS86_1	SEQ ID NO:57

- 20 The design of the oligonucleotide probe should preferably follow these parameters:
- (a) It should be designed to an area of the sequence which has the fewest ambiguous bases ("N's"), if any;
  - (b) It should be designed to have a  $T_m$  of approx. 80 ° C (assuming 2° for each A or T and 4 degrees for each G or C).
- 25 The oligonucleotide should preferably be labeled with  $g\text{-}^{32}\text{P}$  ATP (specific activity 6000 Ci/mmole) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other labeling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in
- 30 a scintillation counter. Preferably, specific activity of the resulting probe should be approximately  $4\text{e}+6$  dpm/pmole.

- The bacterial culture containing the pool of full-length clones should preferably be thawed and 100  $\mu\text{l}$  of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100  $\mu\text{g}/\text{ml}$ . The culture should preferably be grown to
- 35 saturation at 37°C, and the saturated culture should preferably be diluted in fresh L-broth.

Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100 µg/ml and agar at 1.5% in a 150 mm petri dish when grown overnight at 37°C. Other known methods of obtaining  
5 distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them.

The filter is then preferably incubated at 65°C for 1 hour with gentle agitation in 6X SSC (20X stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH)  
10 containing 0.5% SDS, 100 µg/ml of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to 1e+6 dpm/mL. The filter is then preferably incubated at 65°C with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2X SSC/0.5%  
15 SDS at room temperature without agitation, preferably followed by 500 mL of 2X SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1X SSC/0.5% SDS at 65°C for 30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed.

The positive colonies are picked, grown in culture, and plasmid DNA isolated using  
20 standard procedures. The clones can then be verified by restriction analysis, hybridization analysis, or DNA sequencing.

Fragments of the proteins of the present invention which are capable of exhibiting  
25 biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, *Bio/Technology* 10, 773-778 (1992) and in R.S. McDowell, *et al.*, *J. Amer. Chem. Soc.* 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many  
30 purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein  
35 of the invention.

The present invention also provides both full-length and mature forms of the disclosed proteins. The full-length form of the such proteins is identified in the sequence listing by translation of the nucleotide sequence of each disclosed clone. The mature form of such protein may be obtained by expression of the disclosed full-length polynucleotide (preferably those deposited with ATCC) in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein may also be determinable from the amino acid sequence of the full-length form.

The present invention also provides genes corresponding to the cDNA sequences disclosed herein. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials.

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides.

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.



A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived  
5 from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or  
10 any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such  
15 covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego,  
20 California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under  
25 culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-  
30 agarose, heparin-toyopcarl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of  
35 maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits

for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, MA), Pharmacia (Piscataway, NJ) and InVitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from

5 Kodak (New Haven, CT).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Patent No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

### USES AND BIOLOGICAL ACTIVITY

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

#### Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput

screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

#### Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

#### Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one

of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1. 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., *J. Immunol.* 137:3494-3500, 1986; Bertagnolli et al., *J. Immunol.* 145:1706-1712, 1990; Bertagnolli et al., *Cellular Immunology* 133:327-341, 1991; Bertagnolli, et al., *J. Immunol.* 149:3778-3783, 1992; Bowman et al., *J. Immunol.* 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*, J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto, 1994; and Measurement of mouse and human Interferon  $\gamma$ , Schreiber, R.D. In *Current Protocols in Immunology*, J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto, 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*, J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto, 1991; deVries et al., *J. Exp. Med.* 173:1205-1211, 1991; Moreau et al., *Nature* 336:690-692, 1988; Greenberger et al., *Proc. Natl. Acad. Sci. U.S.A.* 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In *Current Protocols in Immunology*, J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto, 1991; Smith et al., *Proc. Natl. Acad. Sci. U.S.A.* 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*, J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto, 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*, J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto, 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring

proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

#### Immune Stimulating or Suppressing Activity

10 A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by

inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected



with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I  $\alpha$  chain protein and  $\beta_2$  microglobulin protein or an MHC class II  $\alpha$  chain protein and an MHC class II  $\beta$  chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492,

1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowman et al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

- 5 Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 10 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

- Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-15 Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

- Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in:20 Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical25 Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

- Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry30 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

- Assays for proteins that influence early steps of T-cell commitment and development35 include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine

et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

#### Hematopoiesis Regulating Activity

5 A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating  
10 utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and  
15 proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell  
20 disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

25 The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others,  
30 proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in:  
35 Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*.

R.I. Freshney, *et al.* eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

#### Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. *De novo* bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears,

deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to

allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions  
5 resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the  
10 following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium ).

15 Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

#### 20 Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in  
25 heterodimers with a member of the inhibin  $\alpha$  family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin- $\beta$  group, may be useful as a fertility  
30 inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., *Endocrinology* 91:562-572, 1972; Ling et al., *Nature* 321:779-782, 1986; Vale et al., *Nature* 321:776-779, 1986; Mason et al., *Nature* 318:659-663, 1985; Forage et al., *Proc. Natl. Acad. Sci. USA* 83:3091-3095, 1986.

#### Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: *Current Protocols in Immunology*, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. *J. Clin. Invest.* 95:1370-1376, 1995; Lind et al. *APMIS* 103:140-146, 1995; Muller et al. *Eur. J. Immunol.* 25: 1744-1748; Gruber et al. *J. of Immunol.* 152:5860-5867, 1994; Johnston et al. *J. of Immunol.* 153: 1762-1768, 1994.

#### Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

#### Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med.



168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltzenberg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

#### Anti-Inflammatory Activity

5 Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production  
10 of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection,  
15 nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

#### Tumor Inhibition Activity

20 In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by  
25 inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

#### Other Activities

30 A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing  
35 or enhancing) bodily characteristics, including, without limitation, height, weight, hair color,

eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

#### ADMINISTRATION AND DOSING

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin.

The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or complement its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent

to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical  
5 compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes  
10 will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or  
15 with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in  
20 which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such  
25 liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show  
30 a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in  
35 combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical

composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01  $\mu$ g to about 100 mg (preferably about 0.1  $\mu$ g to about 10 mg, more preferably about 0.1  $\mu$ g to about 1 mg) of protein of the present invention per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In

the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose

or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, 5 hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on 10 total formulation weight, which represents the amount necessary to prevent desorption of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents 15 beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. 20 Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention.

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the 25 site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth 30 factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a 35 mammalian subject. Polynucleotides of the invention may also be administered by other

known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells  
5 can then be introduced *in vivo* for therapeutic purposes.

Patent and literature references cited herein are incorporated by reference as if fully set forth.



## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Jacobs, Kenneth  
McCoy, John  
LaVallie, Edward  
Racie, Lisa  
Merberg, David  
Treacy, Maurice  
Evans, Cheryl  
Bowman, Michael  
Spaulding, Vikki
- (ii) TITLE OF INVENTION: SECRETED PROTEINS AND POLYNUCLEOTIDES  
ENCODING THEM
- (iii) NUMBER OF SEQUENCES: 57
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Genetics Institute, Inc.
  - (B) STREET: 87 CambridgePark Drive
  - (C) CITY: Cambridge
  - (D) STATE: Massachusetts
  - (E) COUNTRY: U.S.A.
  - (F) ZIP: 02140
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Brown, Scott A.
  - (B) REGISTRATION NUMBER: 32,724
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (617) 498-8224
  - (B) TELEFAX: (617) 876-5851

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 270 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTTCNTGNNG CAAAAACAG AAAACTGGGG NTNNGAAACG TGGGCAGTGT GGTCTTNGNG	60
NGCACTGTAA ATTTGACTTT GTTTNTNTCA CTGAGCGCAT CAGGNATGTC NATTNGGANG	120
GGGGATCNAC ATTCNGGTCC ACAGATACNG ATCTCGGCTT GGGGCGGTCC TCCTCCTGCT	180
GNTGCAGCAA ANCGGGAACC GCGGCCATGG CGACGCGGGA CTCGAGCAGG GCCCGCCTGG	240
CTGTGCGAGG AAAGTAGGCC ATGAAGGCCG	270

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 506 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TANACTTTCC TTAGTTTTTC AAATAGCTCA NCAACCTTCA ATANNTACTT TTTGATGACT	60
GTGGNNTCTT NTAAANCCAG GNTATGTAAN CAGGCTNCGG TGTGNATATA TTCATCGNCA	120
ACATTTNTAT AAAATCTGKT CATTTTTCAT GCTTTMACAC AAGAATCTTT GATCCTATTG	180
TAATAGTTAA TAAGGAAGTT CTTCTCTTGC TCAAAGAAGT CATCTACCTC CTTAACTCCA	240
GTAAAAAGGA CTTTCATCAGC ACTTTTCACC ACACTTTTGA AGAAGCCACC AAACATTTYT	300
TTAGTATTTT TCCGCCTAAC ACTTAGATCC TGATCATATT CCAGGAAAAC ATGAAAGTTG	360
CGATCTTTAC TGAGAACAGG GTGAGAAGAA AGCCGCTGAA GAAAGACTTT CATGGGAGGA	420
CACAGTTYTT CTAAACACA GCGAGATACT CAGCTTCCAG TTCTTGTTTC ATCTGGGCTT	480
ATTATTCCAC CTTCTCCCAG TTTCTG	506

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Lys Val Ala Ile Phe Thr Glu Asn Arg Val Arg Arg Lys Pro Leu
1           5           10           15

Lys Lys Asp Phe His Gly Arg Thr Gln Phe Phe Leu Asn Thr Ala Arg
          20           25           30

Tyr Ser Ala Ser Ser Ser Cys Phe Ile Trp Ala Tyr Tyr Ser Thr Phe
          35           40           45

Ser Gln Phe
          50

```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 85 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

TTTTTAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 60
AAAAAAAAAA AAAAAAAAAA AAAAAA                                     85

```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 517 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

CAATTGCAGA NTTNGAATTC GGCTTTCATG GCATACGGCY TTCATGGCCT AGGGGAGGAA 60

```

GTTGCCTTGT ACTGTGCCAA ATATCTTCCT GATATCATCA AAGATCAGAA GGCCTACAAG 120  
 GAAGGCAAGC TACAGAAGGC TTTAGAAGAT GCCTTCTTGG CTATTGACGC CAAATTGACC 180  
 ACTGAAGAAN TCATTAAAGA GCTGGCACAG ATTGCAGGGC GACCCACTGA GGATGAAGAT 240  
 GAAAAAGAAA AAGTAGCTGA TGAAGATGAT GTGGACAATG AGGAGGCTGC ACTGCTGCAT 300  
 GAAGAGGCTA CCATGACTAT TGAAGAGCTG CTGACACGCT ACGGGCAGAA CTGTCACAAG 360  
 GGCCCTCCCC ACAGCAAATN TGGAGGTGGG ACAGGCGAGG AACCAGGGTC CCAGGGCCTC 420  
 AATGGGGAGG CAGGACCTGA GGA CTCAACT AGGGAACTC CTTACAAGA AAATGGCCCC 480  
 ACAGCCAAGG CCTACACAGG CTTTTCCTCC AACTCGG 517

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 115 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Ala	Tyr	Gly	Xaa	His	Gly	Leu	Gly	Glu	Glu	Val	Ala	Leu	Tyr	Cys	1	5	10	15
Ala	Lys	Tyr	Leu	Pro	Asp	Ile	Ile	Lys	Asp	Gln	Lys	Ala	Tyr	Lys	Glu	20	25	30	
Gly	Lys	Leu	Gln	Lys	Ala	Leu	Glu	Asp	Ala	Phe	Leu	Ala	Ile	Asp	Ala	35	40	45	
Lys	Leu	Thr	Thr	Glu	Glu	Xaa	Ile	Lys	Glu	Leu	Ala	Gln	Ile	Ala	Gly	50	55	60	
Arg	Pro	Thr	Glu	Asp	Glu	Asp	Glu	Lys	Glu	Lys	Val	Ala	Asp	Glu	Asp	65	70	75	80
Asp	Val	Asp	Asn	Glu	Glu	Ala	Ala	Leu	Leu	His	Glu	Glu	Ala	Thr	Met	85	90	95	
Thr	Ile	Glu	Glu	Leu	Leu	Thr	Arg	Tyr	Gly	Gln	Asn	Cys	His	Lys	Gly	100	105	110	
Pro	Pro	His														115			

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 406 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

TTAGATTGTT TTNNGGCCTT CNGGACCTGA GATTGAGTTT TTTTTTTTTC CTTTAGCNTT      60
AGCAGTGGGN ATGAGGTGNG CAGGGGGAGN TGGGTGGTTN AATCCGCCCA TTCCAAAGAG      120
GGTTNTCCTT CNANANTGCA GCNNGGAGCT TTTGANGTCN TTCCAGCCG CTTTGTTCN      180
TNGGGTTNAT NACCGGTTNT GNGCCTGTGT TNTGTTGTGT TGGAGGGAAG GACTGGCGGT      240
TCTGGTTTTT ACTCTGTGAA CTTTATTTAA GGACATTTTT TTTTATTGGG GGTCCATGG      300
CCCTCGGCCG CTKGCACCCG YTTTTTGTG WACACTTTCA ATCAACACTT TTTCAGANTA      360
AAGGCCAAAA CCTAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAA      406

```

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 629 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

CAGTTTTAGA AAGAAGCACC TTGCTGGATA GATTGAGG TTTCTTTTG GAAATTCANA      60
TTCCATATGT GTTTTTTGCA TCTGAAGGAC TTCTTAATAC TCCAGACATA CTTCAGCTGC      120
TAGAATCCAA CTATAACATC TCACTAGTAG AGAGAGGCTG CAGTGAGTCA TTGAAACTCT      180
TTGGAAGTTC AGAGTGTAT GTAGTGGTGA CAATTGATGA ACACACTGCC ATAATTTTGC      240
AGGATCTARA AGAATTGAAT TGTGAGAAGG CATCAGACAA TATCATTATG AGGCTGATGG      300
CATTATCATT ACAGTACAGA TATTGTTGGA TAATTTTATA TACCAAAGAA ACATTAAATT      360

```

CAGAGTATCC GCTTACAGAA AAGACACTTC ATCACCTAGC ACTGATTTAT GCAGCTTTGG 420  
 TTTCATTTGG GCTAAACTCT GAAGAACTGG ATGTAAAGCT TATAATTGCC CCAGGAGTAG 480  
 AAGCAACTGC CTTGATAATT CGACAAATTG CTGACCACAG TTTAATGACC TCAAAGAGAG 540  
 ATCCTCATGA ATGGTTGGAT AAATCCTGGC TTAAAGTTTC ACCATCTGAG GAAGAAATGT 600  
 ACTTACTTGA TTTTNCCATG TATTAACCC 629

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 109 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Arg Leu Met Ala Leu Ser Leu Gln Tyr Arg Tyr Cys Trp Ile Ile  
 1 5 10 15  
 Leu Tyr Thr Lys Glu Thr Leu Asn Ser Glu Tyr Pro Leu Thr Glu Lys  
 20 25 30  
 Thr Leu His His Leu Ala Leu Ile Tyr Ala Ala Leu Val Ser Phe Gly  
 35 40 45  
 Leu Asn Ser Glu Glu Leu Asp Val Lys Leu Ile Ile Ala Pro Gly Val  
 50 55 60  
 Glu Ala Thr Ala Leu Ile Ile Arg Gln Ile Ala Asp His Ser Leu Met  
 65 70 75 80  
 Thr Ser Lys Arg Asp Pro His Glu Trp Leu Asp Lys Ser Trp Leu Lys  
 85 90 95  
 Val Ser Pro Ser Glu Glu Glu Met Tyr Leu Leu Asp Phe  
 100 105

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 280 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCAAGAAAGT TAAAACTTAG GACAAAATNG AAGTTNGAAA ATTTCCAAC TAAAGTATCA	60
TTTTCTGTAA ACACAATTTA AGAACAAATT ANTAAGAGGA AATATTTGCA ACCCAGATAA	120
TAGGAAAAAA AGTTNACATT TNTCATATAT AAAGAATTCC TACAAATTGA TAGAAAGAAG	180
ACAACNTGAT AGAAGAACGG GCAAAATATA TGAACAGATA TTTCTCAGA AAAAAACAAA	240
AATTGTCAAT AAACATTTGA AACACAAAAA AAAAAAAAAA	280

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 298 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGAAWAAGAA GAGAAAGCCA AGGAAGACAA GGGCAAACAA AAGTTGAGGC AGCTTCACAC	60
ACACAGATAC GGAGAACCAG AAGTGCCAGA GTCAGCATTC TGGAAGAAAA TCATAGCATA	120
TCAACAGAAA CTTCTAAACT ATTTTGCTCG CAACTTTTAC AACATGAGAA TGTTAGCCTT	180
ATTTGTCGCA TTTGCTATCA ATTCATCTT GCTCTTTTAT AAGGTCTCCA CTTCTTCTGT	240
GGTTGAAGGA AAGGAGCTCC CCACGAGAAG TTCAAGTGAA AATGCCAAAG TGACAAGC	298

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

(2) INFORMATION FOR SEQ ID NO:13:

(A) LENGTH: 165 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

(2) INFORMATION FOR SEQ ID NO:14:

(A) LENGTH: 224 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

(2) INFORMATION FOR SEQ ID NO:15:

(A) LENGTH: 595 base pairs



(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```

CTGGCCGCCA GGTAGAGCGT TGGTTCCGTC GCCGCCGCAA CCAGGACCGG CCCAGTNTCN      60
TCAAGAAGTT CCGAGAAGCC AGCTGGAGAT TCACATTTTA CNTGATTGCN TTCATTGCCG      120
GCATGGCCGT CATTGTGGAT AAACCCTGGT TCTATGACAT GAAGAAAGTT TGGGAGGGAT      180
ATCCCATACA GAGCACTATC CNTTCCCAGT ATTGGTACNA CATGATTGAA CTTTCCTTNT      240
ACTGGTGCSG TGCTCTTCAG CATTGCCTCT GATGTCAAGC GAAAGGATTT CAAGGAACAG      300
ATCATCCACC ATGTGKCCAC CATCATCTCT ATCAGCTTTT CCTGGGTTTG CCAATTACAT      360
CCGAGCTGGG ACTCTAATCA TGGCTCTGCA TGACTCTTCC GATTACCTGC TGGAGTCAGC      420
CAAGATGTTT AACTACGCGG GATGGAAGAA CACCTGCAAC AACATCTTCA TCGTCTTCGC      480
CATTGTTTTT ATCATACCCC GACTGGTCNT CCTGCCCTTC TGGNTCCTGC ATTGCACCCT      540
GGTGTNCCCN CTGGAGCTCT ATCCTGCCTT CTTTGGCTNT TACTTCTTCN ATTCC          595
  
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 129 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

Ser Ser Ala Leu Pro Leu Met Ser Ser Glu Arg Ile Ser Arg Asn Arg
1           5           10           15
Ser Ser Thr Met Xaa Pro Pro Ser Phe Ser Ser Ala Phe Pro Gly Phe
20          25          30
Ala Asn Tyr Ile Arg Ala Gly Thr Leu Ile Met Ala Leu His Asp Ser
35          40          45
  
```

Ser Asp Tyr Leu Leu Glu Ser Ala Lys Met Phe Asn Tyr Ala Gly Trp  
 50 55 60

Lys Asn Thr Cys Asn Asn Ile Phe Ile Val Phe Ala Ile Val Phe Ile  
 65 70 75 80

Ile Thr Arg Leu Val Ile Leu Pro Phe Trp Ile Leu His Cys Thr Leu  
 85 90 95

Val Tyr Pro Leu Glu Leu Tyr Pro Ala Phe Phe Gly Tyr Tyr Phe Phe  
 100 105 110

Asn Ser Met Met Gly Val Leu Gln Leu Leu His Ile Phe Trp Ala Tyr  
 115 120 125

Leu

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 145 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGTTTTAAAG CCAGAATTAC GGNTAGCACC TAGCATTTC A GCAGAGGGAC CATTTTAGAC 60

CAAAATGTAC TGTTAANGGG TTTT TTTTAA AAATTAAAAG ATTAAATAAA AAATATTAAA 120

TAAACANGA AAAAAAAAAA AAAAA 145

## (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 398 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAATTCGGCC AAANANGCCT AGGCAGAATG GGA CTCCAAG CCTGCCTCCT AGGGCTCTTT 60

```

GCCCTCATCC TCTCTGGCAA ATGCAGTTAC AGCCCGGAGC CCGACCAGCG GAGGACGCTG      120
CCCCCAGGCT GGGTGTCCCT GGGCCGTGCG GACCCTGAGG AAGAGCTGAG TCTCACCTTT      180
GCCCTGAGAC AGCAGAATGT GGAAAGACTC TCGGAGCTGG TGCANGCTGT GTCGGATCCC      240
AGCTCTCCTC AATACGGAAA ATACCTGACC CTAGAAAAAT GTGGCTGATC TGGTGAGGCC      300
ATCCCCACTG ACCCTCCACA CGGTGCAAAA ATGGCTCTTG GCAGCCCGGA NCCCAAAAAT      360
TGCCATTCTG TGATCACACA GGAACCTTCT GACTTGCT                                398

```

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

Met Gly Leu Gln Ala Cys Leu Leu Gly Leu Phe Ala Leu Ile Leu Ser
1           5           10           15
Gly Lys Cys Ser Tyr Ser Pro Glu Pro Asp Gln Arg Arg Thr Leu Pro
20           25           30
Pro Gly Trp Val Ser Leu Gly Arg Ala Asp Pro Glu Glu Glu Leu Ser
35           40           45
Leu Thr Phe Ala Leu Arg Gln Gln Asn Val
50           55

```

## (2) INFORMATION FOR SEQ ID NO:20:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 437 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

TGCTTCCTTG GGGGTGAGTT TTCATTCACT ATGTGGGGAG GGACCTTACG GGAAAACCCA      60

```

```

TGTGTGAAGG TTCCCCAAA TTCCTACCAG CTTTCCACAG GCCTTGGCCC CCCATGTGGA    120
CTTTGTGGGG GGACTGCACC GTTTTTC CCC CAACATCATC CCTGAGGCAA CGTCCTGAGC    180
CGCAGGTGAC AGGGACTGTA GGCCTGCATC TGGGGGTAAC CCCCTCTGTG ATCCGTAAGC    240
GATACAAC TT GACCTCACAA GACGTGGGCT CTGGCACCAG CAATAACAGC CAAGCCTGTG    300
CCCAAGTTCCT GGAGCAGTAT TTCCATGACT CAGACCTGGC TCAGTTCATG CGCCTCTTCG    360
CTGGCAAC TT TGCACATCAG GCATCAGTAG CCCGTGTGGT TGGACAACAG GGCCGGGGCC    420
GGCCGGCGCA TCTCGAG                                                    437

```

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 419 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

CGGGGACCWA GTGGCAACGA CTTGGACATC TGAGCTGTCA CTGCCGAAAA CAGGCCGCAA    60
GAGAGATAAT CAATATGCAT TTCCAAGCCT TTTGGCTATG TTTGGGTCTT CTGTTCATCT    120
CAATTAATGC AGAATTTATG GATGATGATG TTGAGACGGA AGACTTTGAA GAAAATTCAG    180
AAGAAATTGA TGTTAATGAA AGTGAAC TTT CCTCAGAGAT TAAATATAAG ACACCTCAAC    240
CTATAGGAGA AGTATATTTT GCAGAAACTT TTGATAGTGG AAGGTTGGCT GGATGGGTCT    300
TATCMAAARC AAAGAAAGAT GACATGGATG AGGAAATTTT AATATWCGAT GGAAGATGGG    360
AAATTGAAGA GTTGAMP.GAA AACCAGGTAC CTGGTGACAG AGGACTGGTA TTAAAATCT    419

```

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 57 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met His Phe Gln Ala Phe Trp Leu Cys Leu Gly Leu Leu Phe Ile Ser  
 1 5 10 15  
 Ile Asn Ala Glu Phe Met Asp Asp Asp Val Glu Thr Glu Asp Phe Glu  
 20 25 30  
 Glu Asn Ser Glu Glu Ile Asp Val Asn Glu Ser Glu Leu Ser Ser Glu  
 35 40 45  
 Ile Lys Tyr Lys Thr Pro Gln Pro Ile  
 50 55

## (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 94 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 60  
 AAAAAAAAAA AAAAAAAAAA AAAAAAGCGG CCGC 94

## (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 399 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CAGAGAGGCT GAGACCAACC CAGAAACCAC CACYTCTCAC GCCAAAGCTC ACACCTTCAG 60  
 CCTCCAACAT GAAGGTCTCC GCAGCACTTC TGTGGCTGCT GTCATAGCA GTGCTTCA 120  
 GCCCCAGGG GCTCGCTGGG CCAGCTTCTG TCCAACCAC CTGCTGCTTT AACCTGGCCA 180  
 ATAGGAAGAT ACCCCTTCAG CGACTAGAGA GCTACAGGAG AATCACCAGT GGCAAATGTC 240

CCCAGAAAGC TGTGATCTTC AAGACCAAAC TGGCCAAGGA TATHTGTGCC GACCCCAAGA 300  
 AGAAGTGGGT GCAGGATTCC CATGAAGTAT CTGGACCAAA AATCTCCAAC TCCAAAGCCA 360  
 TAAATAATCA CCATTTTNGA AACCAAAAAA AAAAAAAAAA 399

## (2) INFORMATION FOR SEQ ID NO:25:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 82 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Lys Val Ser Ala Ala Leu Leu Trp Leu Leu Leu Ile Ala Xaa Ala  
 1 5 10 15  
 Phe Ser Pro Gln Gly Leu Ala Gly Pro Ala Ser Val Pro Thr Thr Cys  
 20 25 30  
 Cys Phe Asn Leu Ala Asn Arg Lys Ile Pro Leu Gln Arg Leu Glu Ser  
 35 40 45  
 Tyr Arg Arg Ile Thr Ser Gly Lys Cys Pro Gln Lys Ala Val Ile Phe  
 50 55 60  
 Lys Thr Lys Leu Ala Lys Asp Ile Cys Ala Asp Pro Lys Lys Lys Trp  
 65 70 75 80  
 Val Gln

## (2) INFORMATION FOR SEQ ID NO:26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 448 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATTTTGAAT TCGGCCAAAG AGGCCTACAA GGGACTAGCA GGCCTAGGGA TACCCTTCCT 60

```

CTATGGCTCC AGTGTCCCAG CTGCCCCCGC TGCCTACCAT GGCAGGAGCA TGCTCCCTGC      120
CGGTGACCTG CATTTTCACA GAAGCACCK CAGAAACCTT CAGGGAAACC CCATGCTAGC      180
GGCAACTGCA CCACACTTTG AGGAGAGCTG GGGGCAGAGA TGTNGTCGAC TCAGGAAAAA      240
TACAGGGAAT CAAAAGCTC TAGACAGTGA TGCTGAGAGT TCCAAAAGTC AAGCAGAAGA      300
AAAAATCCTA GGTCAGACTT ATGCAGTTCC CTATGAAGAC GATCATTATG CAAAAGACCC      360
AGACATTGAA GCACCCAGCA ACCAGAAGTC AAGTGAAACG AATGAAAAGC CAACGACAGC      420
TCTTGCCAAC ACCTGTGGAG AGCTCGAG                                         448

```

## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 113 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```

Met Leu Pro Ala Gly Asp Leu His Phe His Arg Ser Thr Xaa Arg Asn
1           5           10           15
Leu Gln Gly Asn Pro Met Leu Ala Ala Thr Ala Pro His Phe Glu Glu
20          25          30
Ser Trp Gly Gln Arg Cys Xaa Arg Leu Arg Lys Asn Thr Gly Asn Gln
35          40          45
Lys Ala Leu Asp Ser Asp Ala Glu Ser Ser Lys Ser Gln Ala Glu Glu
50          55          60
Lys Ile Leu Gly Gln Thr Tyr Ala Val Pro Tyr Glu Asp Asp His Tyr
65          70          75          80
Ala Lys Asp Pro Asp Ile Glu Ala Pro Ser Asn Gln Lys Ser Ser Glu
85          90          95
Thr Asn Glu Lys Pro Thr Thr Ala Leu Ala Asn Thr Cys Gly Glu Leu
100         105         110
Glu

```

## (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 287 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGCTCACGGC TGTAATCCCA ACACTTTGGG AGTCTGAGGC GGGNGAATCA TGAGGTCAGG	60
AGATTAAAGAC CAGCTTGGCC AACATGGTGA AACCCCGTNT NTACTAAAAA TACAAAAAAA	120
TTAGCTAGGC CTGGTGGTGC GCGAATGTAG TCCCAGCTAC TCGGGAGGCT GAAGCAGGAG	180
AATTGCTTGA ACCTGGGAAG CGGAGGCTAC AGTGAGCTGA GATCGTGCCA CTGCACTCCA	240
GCCTGGGTGA CAGAGCAGGA CTCTGTNTCA AAAAAAAAAA AAAAAAA	287

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 228 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGANAGCTGT TCAGCTATGG GTGCTGTCAC ANATTANCTG TATCTTTGNC AGTTTAGCTG	60
GATGCTCATT CAGTCTGTGA ATTTCTGGTA NGTGCTGGTG ATGAATGATG AGCACACAGA	120
GAGGGGATAT CTGCTGTTTT TCCTTCTGAG TTGGGGANTA CCAGCTTTTG TGGTGATTCT	180
CCTCATAGTT ATTTTGAAAG GAATCTATCA TCAGAGCATG TCANAGAT	228

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 541 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA



- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 467 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```

GCAGGAAATC TGGGGATCTA AATCCAGAAT GATTCAGAGA GCTGGAGGAA GGGGGCTGCC      60
CTGGCTTAAC TTGGTTCATT CCCAGGCCAA CACCTCACCG TGATCCACGT CCCCACTGCT      120
GTGCTGAAGC TGGNGTNTGC CCCAGGGAAC CCTGCCGGTC ACACATGYTC AGGATTTTCAT      180
GGGCCTGTGT CNACCCTGCT TTTTCTTTA TTCTTNGTAG TNGTTTAGGA GTGGGGGGCC      240
TCGCAGAACA CNTAGTCCAG CCCACTGCCC AGAGCAGGTG TGTCCCTTTC ATAMTTCAGT      300
CCACTTTAAA ACAGCCTTCC CCCACCCCTT TYTATGGTAG CAGTTYTCCT CGGGGTYTCC      360
ATGGACACCC TGTGCCCCAA GCCGATGGCC CCACCCAGCA GCATCAGCAC AGCTGCCCCC      420
CTTYTCCGCA GAGCAGGCTY TCCTTTACGG GAMTYTCCTY TTCCCTC                    467
  
```

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 28 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```

Phe Phe Phe Ile Leu Xaa Ser Xaa Leu Gly Val Gly Gly Leu Ala Glu
1           5           10          15
His Xaa Val Gln Pro Thr Ala Gln Ser Arg Cys Val
          20          25
  
```

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 279 base pairs  
 (B) TYPE: nucleic acid

- (C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```

GGGAGGCTGA NGCATAAGGA TCACTTGAGC CCAGGAGTTC ANGACCAGCC TAACATAGTG      60
AGACCCCTGA NTTTACAAAA AATTAAAGTT AGCCAGGTGT GGTGGNACAN GCCTGTGGTC      120
CCAGCTACAC AGGAAGCTGA GGCAGGAGGA TATNTTGAGC CTAGGAATTC AAGGCTGCAG      180
NGAGCTGTGA TNACACCACT GAACTCCTGA ACTCCAGTGT GCGTGACAGA GCAAGACTCT      240
GTNTCNAAAN AAAAANAAAA AAAAAAAAAA GCGGCCGCT      279

```

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 233 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```

AAGCCCCCA CGCCCCGAGC CCACCCTGCT CACCGGCCTC TGCCCGAGTT CCCC GCATAG      60
TGTGGGAGTG TGGANCATCC TANCTTTTCC CCAGCGCCCA GTTCTTTCAC TCTCACTGGA      120
GTCCTGCAGG GACAGCTCGG GCACCATGTA NGCCCGGGTG GGCGTGGGGG CTCACCTANC      180
TCGGTGGTGA ACAGCTGGCA CGTCTCTGGG TTGCGGACGG TAAAGGCCAC GTA      233

```

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 579 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```

GACCTGTNTT TTATTCCAAA CGTCTATNCT GCTTTNTTCA CTGCAGCTNC TTGTTCTTTT      60
GACNTNCCTC NTGNTGGTNT TCNTGGTNTT CATCCATGCC TACCAGGTGA ANCCACANTG      120
GAAAGCATAT GATGATNTNT TCAGAGGAAG GACAAATGCT GCAGAAATTC CACTGATTTT      180
ATATCTCTTT GCTCTGATTT CCNTGACATG GCTTTGGGGA GGA CTACACA TGGCCTACAG      240
GCACTTCTGG ATGTTGGTTC TCTTTGTCAT TTTCAACAGT CTGCAGGGAC TTTATGTTTT      300
CATGGTTTAT TTCATTTTWC ACAACCAAAT GTGTTGCCCT ATGAAGGCCA GTTACACTGT      360
GGAAATGAAT GGGCATCCTG GACCCAGCAC AGCCTTTTTC ACGCCCGGGA GTGGAATGCC      420
TCCTGCTGGA GGGGAAATCA GCAAGTCCAC CCAGAATCTC ATCGGTGCTA TGGAGGAGGT      480
GCCACCTGAC TGGGAGAGAG CATCCTTCCA ACAGGGCAGT CAGGCCAGCC CTGATTTAAA      540
G                                                                           541

```

## (2) INFORMATION FOR SEQ ID NO:31:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```

Met Ala Tyr Arg His Phe Trp Met Leu Val Leu Phe Val Ile Phe Asn
1           5           10           15
Ser Leu Gln Gly Leu Tyr Val Phe Met Val Tyr Phe Ile Xaa His Asn
20          25          30
Gln Met Cys Cys Pro Met Lys Ala Ser Tyr Thr Val Glu Met Asn Gly
35          40          45
His Pro Gly Pro Ser Thr Ala Phe Phe Thr Pro Gly Ser Gly Met Pro
50          55          60
Pro Ala Gly Gly Glu Ile Ser Lys Ser Thr Gln Asn Leu Ile Gly Ala
65          70          75          80

```

Met Glu Glu Val Pro Pro Asp Trp Glu Arg Ala Ser Phe Gln Gln Gly  
                             85                            90                            95

Ser Gln Ala Ser Pro Asp Leu Lys  
                             100

## (2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 238 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CCTGTGAATT NTACTGGATG ATTAATACAA ACGTGATTGT TGTATTTGGA GTATAAATTA	60
CTGATTGTAT GTNACCTGAA AATTCAC TGC TATAAGAAAG GTGGANTCAG TTTGTATCAN	120
TTAATAGGAT TTTCATATTC CAAGGATATT AGTTGTTTTT TTAATCATCC TATATGGCTA	180
ACATTGTTTA ATGAAAGTAA TAATCAATAA AGCAATAGAA TCTAAAAAAA AAAAAAAA	238

## (2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 265 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TTGCTGTTGC GCGGGATGGT GGATGACTTG TCAAAC TCGG GCGGGCCCTC GCTGTCCGCA	60
TCCCCATTCA CGGAGTANCA NTCGTANTCN GAGCCTGGGG GCACGGGACA CANTGAGGCC	120
CANGGCCCAN GTGGCCCCCTT GCCCCCAGCC CACCAGGGTG AGCACAGAGG GGGAAGGACG	180
GGGCCCTCCT GGATGGCTAA NTCCCANCTG TCCCTGGTCC CACCCCANCC CCGCGGGCCT	240
GCCTTGGGAA GGGATGGTGT CCTCA	265

## (2) INFORMATION FOR SEQ ID NO:34:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```

ACGNGNGNTC CATGCCAGA ATTCCNGTNG TNTCAGGAAT GTTCAATAGA TGGAATCCTG      60
TGTGGCCTGA GANGAGTGTT TTTCATGCCG NGTGACACCC TNGAGGCCCG NGCAACTGTT      120
GGTANGTCAA CAGTTAGCTG CTTCTCATTG CNGAGTGGCG ATTGGTCCTG TCATGGTTTA      180
TTCAGCCATG NGGNGGATGG CTA CTGTTT TTAAGCCAC TTGCCTTCTG ATCGCTGGAC      240
NGACTCTYTC GCCYTYTCTT GGTGCAGTCC TCAGGAGGCT CGGTCACAYT CTCCAAGAGC      300
ACAGCCATCA TCTCCCACGG TACCACAGGC CTGGTCACAT GGGATGCCGC CCTYTACCTT      360
GCAGAATGGG CCATCGAGAA CCCGGCAGCC TTCATTAACA GGTGACCTCG GGGCACAGGG      420
CAGGGCACCG AGGCAGGCTT ACCCTGGTGC AGTCGAAAAC ACGGTCCCCT TTCCTCCCGC      480
CAGGACTGTC CTAGAGCTTG GCAGTGGTGC CGGCCTCACA GGCCTTGCCA TYTGCAAGAN      540
GTGCCGCCCC CGGGCATACA TYTTCAGCGA CCCTCACAG                               579

```

## (2) INFORMATION FOR SEQ ID NO:39:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```

Met Val Tyr Ser Ala Met Xaa Xaa Met Ala Thr Cys Phe Xaa Ser His
1           5           10           15
Leu Pro Ser Asp Arg Trp Thr Asp
                20

```

## (2) INFORMATION FOR SEQ ID NO:40:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 87 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 60  
 AAAAAAAAAA AAAAAAAAAAG CGGCCGC 87

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 224 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GCTTTTTTTT TTTTTTTTGT ATCTTTCTAG TTCATTCTGT CCATTGNTAN TTTTTTATAA 60  
 ANAAAATTTT ACTACCATAT ACTTTCTGTT CCANANACAG GAAACTGTTT GCAGGTCCCT 120  
 GAACCTACCT TCATTTTCTA GTGCTGTGCA TTTCCTCATT TCTTTCATTT GGAAANTGGT 180  
 GAAAANGTCC TCTAACTTGC TTCTTGCCCT CATTTCTCTA AGCA 224

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 593 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TGTTCTTCNG GCACTTCCCA TCACCGNGAT AGCATATTTT ACTGGGGTGG TTTCCATTCC 60  
 CATTTCTCT ACTATAGTGA GCCACTGGAG GCGAGAACCC TNGTATTTCC AACATTGGTG 120  
 TACTCAGCAT CTTGCGTCGA GGA CTAGTA AGTATTATAT TTGAATTCCC ACTGCACCGC 180  
 TCTAATTAGA ATTTTAAAAA TCAC TTCTA TGTGGATTGT NACACACTTT TTTTCCCCTT 240  
 AATTCATTTT TCTCCANGNA CTACCCATAT GCATCCTATA TAAATTTACC AGCACTCATA 300  
 AAAATCTTAC TCAGAAATCT TCAGAGGTTT GCTAAGGATA CAATTTGATT CTTACACATT 360

TAATGCTCAC CAGCTGCTTA GGCCACACACC ATTTATCCAC CCTGATTGTC TACTGCTCTT 420  
 TGAAATACAA CCAGTGTTTC AGCCAGACTG TTTTCCTGCT TCTGCTCCCC TTCTCCTCCT 480  
 CCCAGCACAT CTGTGAATTC TTTGACTGGT TTACCACTCC CAMACTCCTC CCCAGCAATG 540  
 CAGATCTTCT ACACCCTTTA GGATCTAAGC TAAGTCTGCT TCCCAGATAT CCT 593

## (2) INFORMATION FOR SEQ ID NO:43:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met	Leu	Thr	Ser	Cys	Leu	Gly	Pro	His	His	Leu	Ser	Thr	Leu	Ile	Cys
1				5						10				15	
Tyr	Cys	Ser	Leu	Lys	Tyr	Asn	Gln	Cys	Phe	Ser	Gln	Thr	Val	Phe	Leu
			20					25					30		
Leu	Leu	Leu	Pro	Phe	Ser	Ser	Ser	Gln	His	Ile	Cys	Glu	Phe	Phe	Asp
		35					40					45			
Trp	Phe	Thr	Thr	Pro	Xaa	Leu	Leu	Pro	Ser	Asn	Ala	Asp	Leu	Leu	His
		50				55					60				
Pro	Leu	Gly	Ser	Lys	Leu	Ser	Leu	Leu	Pro	Arg	Tyr	Pro			
65					70					75					

## (2) INFORMATION FOR SEQ ID NO:44:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 256 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

TCNTGNATTT AATTATTTAA GCTATATTAA AAAAATTGAA ACCCTTAGAT GTTAATTAAT 60

TTTAAAACT ANTGATNGAT GCAGNTAAGC TAGAATGATT GGATCAAATC TCACACACAA 120  
ATGAGTTTAT TCTTTAAAAA AAAATTTTTT TTTTAGAGAC GGNTTCTTGC TATGTTCCCC 180  
AGGATGTTCT TGAATCATG ACCTCAAGCA ATCCTCCTCC CTCACCCTAC CTGAATTAAA 240  
AAAAAAAAAA AAAAAA 256

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AATGATCAGG ATCTAAGTGT TAGGCGGA

28

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GGTTTCCCTA GTTGAGTCCT CAGGTCCT

28

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TATCAAGGCA GTTGCTTCTA CTCCTGGG

28

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ACAAGATGAA ATTGATAGCA AATGCGAC

28

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

ACCTGCTGGA GTCAGCCAAG ATGTTTA

27

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GAATGGGACT CCAAGCCTGC CTCCTA

26

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

TGAGATGAAC AGAAGACCCA AACATAGC

28

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TCTCAGCCA AAGCTCACAC CTTCAGC

27

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TTGGGTCTTT TGCATAATGA TCGTCTTC

28

## (2) INFORMATION FOR SEQ ID NO:54:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GAACTGGCCT TCATAGGGCA ACACATTT

28

## (2) INFORMATION FOR SEQ ID NO:55:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

AACCCCGAGG AGAACTGCTA CCATAGAA

28

## (2) INFORMATION FOR SEQ ID NO:56:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TCTCGATGGC CCATTCTGCA AGGTAGAG

28

## (2) INFORMATION FOR SEQ ID NO:57:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GTTGTATTTC AAAGAGCAGT AGCAAATC

28

What is claimed is:

1. A composition comprising an isolated polynucleotide selected from the group consisting of:
  - (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2;
  - (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 351 to nucleotide 506;
  - (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AZ302\_1 deposited under accession number ATCC 98076;
  - (d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AZ302\_1 deposited under accession number ATCC 98076;
  - (e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AZ302\_1 deposited under accession number ATCC 98076;
  - (f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AZ302\_1 deposited under accession number ATCC 98076;
  - (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:3;
  - (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:3 having biological activity;
  - (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;
  - (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above ; and
  - (k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h).
2. A composition of claim 1 wherein said polynucleotide is operably linked to an expression control sequence.
3. A host cell transformed with a composition of claim 2.
4. The host cell of claim 3, wherein said cell is a mammalian cell.
5. A process for producing a protein, which comprises:

- (a) growing a culture of the host cell of claim 3 in a suitable culture medium;
  - and
  - (b) purifying the protein from the culture
6. A protein produced according to the process of claim 5.
7. The protein of claim 6 comprising a mature protein.
8. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:
- (a) the amino acid sequence of SEQ ID NO:3;
  - (b) fragments of the amino acid sequence of SEQ ID NO:3; and
  - (c) the amino acid sequence encoded by the cDNA insert of clone AZ302\_1
- deposited under accession number ATCC 98076;
- the protein being substantially free from other mammalian proteins.
9. The composition of claim 8, wherein said protein comprises the amino acid sequence of SEQ ID NO:3.
10. The composition of claim 8, wherein said protein comprises the amino acid sequence of SEQ ID NO:3 from amino acid to amino acid .
11. The composition of claim 8, further comprising a pharmaceutically acceptable carrier.
12. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 11.
13. The gene corresponding to the cDNA sequence of SEQ ID NO:2, SEQ ID NO:1 or SEQ ID NO:4 .
14. A composition comprising an isolated polynucleotide selected from the group consisting of:
- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;

- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 23 to nucleotide 517;
- (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AU139\_2 deposited under accession number ATCC 98076;
- (d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AU139\_2 deposited under accession number ATCC 98076;
- (e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AU139\_2 deposited under accession number ATCC 98076;
- (f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AU139\_2 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;
- (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;
- (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;
- (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above ; and
- (k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h).

15. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:6;
- (b) the amino acid sequence of SEQ ID NO:6 from amino acid 35 to amino acid 115;
- (c) fragments of the amino acid sequence of SEQ ID NO:6; and
- (d) the amino acid sequence encoded by the cDNA insert of clone AU139\_2 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins.

16. The gene corresponding to the cDNA sequence of SEQ ID NO:5 or SEQ ID NO:7.

17. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 288 to nucleotide 629;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 441 to nucleotide 629;
- (d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AU105\_14 deposited under accession number ATCC 98076;
- (e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AU105\_14 deposited under accession number ATCC 98076;
- (f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AU105\_14 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AU105\_14 deposited under accession number ATCC 98076;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:9;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:9 having biological activity;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above; and
- (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i) .

18. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:9;
- (b) the amino acid sequence of SEQ ID NO:9 from amino acid 25 to amino acid 44;
- (c) fragments of the amino acid sequence of SEQ ID NO:9; and
- (d) the amino acid sequence encoded by the cDNA insert of clone AU105\_14 deposited under accession number ATCC 98076;



the protein being substantially free from other mammalian proteins.

19. The gene corresponding to the cDNA sequence of SEQ ID NO:8 or SEQ ID NO:10.
20. A composition comprising an isolated polynucleotide selected from the group consisting of:
- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11;
  - (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 164 to nucleotide 298;
  - (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS268\_1 deposited under accession number ATCC 98076;
  - (d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AS268\_1 deposited under accession number ATCC 98076;
  - (e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS268\_1 deposited under accession number ATCC 98076;
  - (f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS268\_1 deposited under accession number ATCC 98076;
  - (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;
  - (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity;
  - (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;
  - (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above ; and
  - (k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h).
21. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:
- (a) the amino acid sequence of SEQ ID NO:12;
  - (b) fragments of the amino acid sequence of SEQ ID NO:12; and

(c) the amino acid sequence encoded by the cDNA insert of clone AS268\_1 deposited under accession number ATCC 98076;  
the protein being substantially free from other mammalian proteins.

22. The gene corresponding to the cDNA sequence of SEQ ID NO:11 or SEQ ID NO:13 .

23. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:21;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:21 from nucleotide 75 to nucleotide 419;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:21 from nucleotide 132 to nucleotide 419;
- (d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AJ147\_1 deposited under accession number ATCC 98076;
- (e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AJ147\_1 deposited under accession number ATCC 98076;
- (f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AJ147\_1 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AJ147\_1 deposited under accession number ATCC 98076;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:22;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:22 having biological activity;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and
- (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

24. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:22;
- (b) fragments of the amino acid sequence of SEQ ID NO:22; and
- (c) the amino acid sequence encoded by the cDNA insert of clone AJ147\_1

deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins.

25. The gene corresponding to the cDNA sequence of SEQ ID NO:21 or SEQ ID NO:23 .

26. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:24;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:24 from nucleotide 69 to nucleotide 377;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:24 from nucleotide 120 to nucleotide 377;
- (d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AM262\_11 deposited under accession number ATCC 98076;
- (e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AM262\_11 deposited under accession number ATCC 98076;
- (f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AM262\_11 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AM262\_11 deposited under accession number ATCC 98076;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:25;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:25 having biological activity;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and

(l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

27. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:25;
- (b) the amino acid sequence of SEQ ID NO:25 from amino acid 14 to amino acid 81;
- (c) fragments of the amino acid sequence of SEQ ID NO:25; and
- (d) the amino acid sequence encoded by the cDNA insert of clone AM262\_11 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins.

28. The gene corresponding to the cDNA sequence of SEQ ID NO:24.

29. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:26;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:26 from nucleotide 110 to nucleotide 448;
- (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AR28\_1 deposited under accession number ATCC 98076;
- (d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AR28\_1 deposited under accession number ATCC 98076;
- (e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR28\_1 deposited under accession number ATCC 98076;
- (f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR28\_1 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:27;
- (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:27 having biological activity;
- (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;

- (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above; and
- (k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h).

30. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:27;
- (b) the amino acid sequence of SEQ ID NO:27 from amino acid 15 to amino acid 78;
- (c) fragments of the amino acid sequence of SEQ ID NO:27; and
- (d) the amino acid sequence encoded by the cDNA insert of clone AR28\_1 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins.

31. The gene corresponding to the cDNA sequence of SEQ ID NO:26 or SEQ ID NO:28.

32. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:30;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:30 from nucleotide 230 to nucleotide 541;
- (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS162\_1 deposited under accession number ATCC 98076;
- (d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AS162\_1 deposited under accession number ATCC 98076;
- (e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS162\_1 deposited under accession number ATCC 98076;
- (f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS162\_1 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:31;

- (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:31 having biological activity;
- (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above; and
- (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above .

33. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:31;
- (b) the amino acid sequence of SEQ ID NO:31 from amino acid 5 to amino acid 25;
- (c) fragments of the amino acid sequence of SEQ ID NO:31; and
- (d) the amino acid sequence encoded by the cDNA insert of clone AS162\_1 deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins.

34. The gene corresponding to the cDNA sequence of SEQ ID NO:30, SEQ ID NO:29 or SEQ ID NO:32 .

35. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:34;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:34 from nucleotide 202 to nucleotide 467;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:34 from nucleotide 241 to nucleotide 467;
- (d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS264\_3 deposited under accession number ATCC 98076;
- (e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AS264\_3 deposited under accession number ATCC 98076;
- (f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS264\_3 deposited under accession number ATCC 98076;

- (g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS264\_3 deposited under accession number ATCC 98076;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:35;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:35 having biological activity;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and
- (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

36. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:35;
- (b) fragments of the amino acid sequence of SEQ ID NO:35; and
- (c) the amino acid sequence encoded by the cDNA insert of clone AS264\_3

deposited under accession number ATCC 98076;  
the protein being substantially free from other mammalian proteins.

37. The gene corresponding to the cDNA sequence of SEQ ID NO:34, SEQ ID NO:33 or SEQ ID NO:36.

38. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:38;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:38 from nucleotide 173 to nucleotide 579;
- (c) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS301\_2 deposited under accession number ATCC 98076;
- (d) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AS301\_2 deposited under accession number ATCC 98076;

- (e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS301\_2 deposited under accession number ATCC 98076;
- (f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS301\_2 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:39;
- (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:39 having biological activity;
- (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;
- (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above ; and
- (k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h).

39. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:39;
- (b) fragments of the amino acid sequence of SEQ ID NO:39; and
- (c) the amino acid sequence encoded by the cDNA insert of clone AS301\_2

deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins.

40. The gene corresponding to the cDNA sequence of SEQ ID NO:38, SEQ ID NO:37 or SEQ ID NO:40 .

41. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:42;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:42 from nucleotide 363 to nucleotide 593;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:42 from nucleotide 483 to nucleotide 593;
- (d) a polynucleotide comprising the nucleotide sequence of the full length protein coding sequence of clone AS86\_1 deposited under accession number ATCC 98076;



- (e) a polynucleotide encoding the full length protein encoded by the cDNA insert of clone AS86\_1 deposited under accession number ATCC 98076;
- (f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS86\_1 deposited under accession number ATCC 98076;
- (g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS86\_1 deposited under accession number ATCC 98076;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:43;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:43 having biological activity;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above; and
- (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

42. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:43;
- (b) fragments of the amino acid sequence of SEQ ID NO:43; and
- (c) the amino acid sequence encoded by the cDNA insert of clone AS86\_1

deposited under accession number ATCC 98076;

the protein being substantially free from other mammalian proteins.

43. The gene corresponding to the cDNA sequence of SEQ ID NO:42, SEQ ID NO:41 or SEQ ID NO:44 .

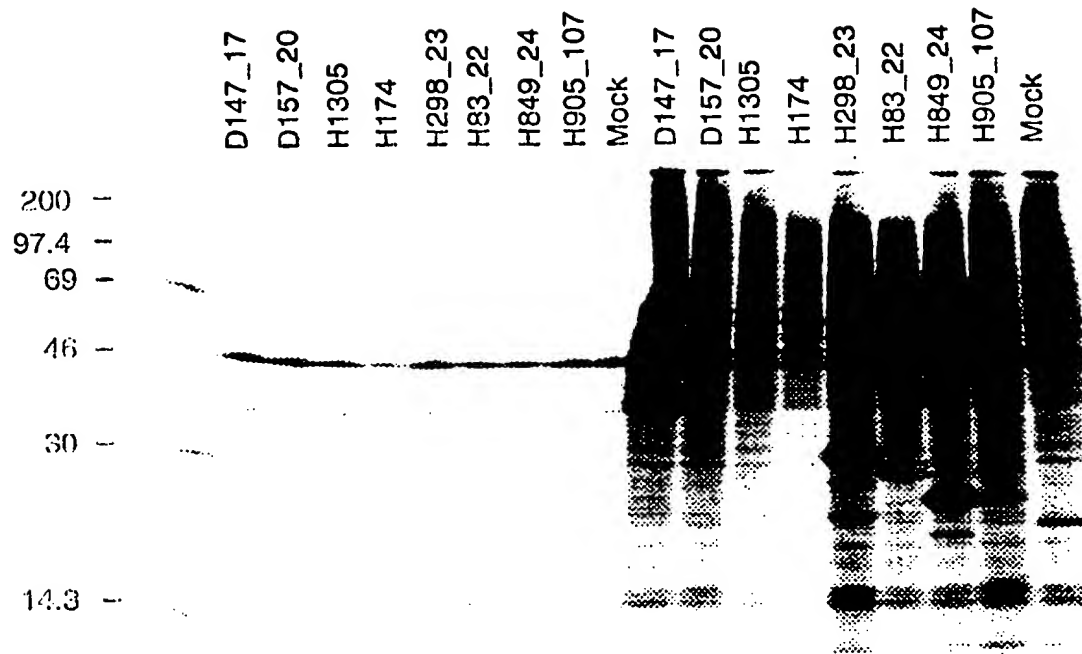


Fig. 1  
1/2

SUBSTITUTE SHEET (RULE 26)

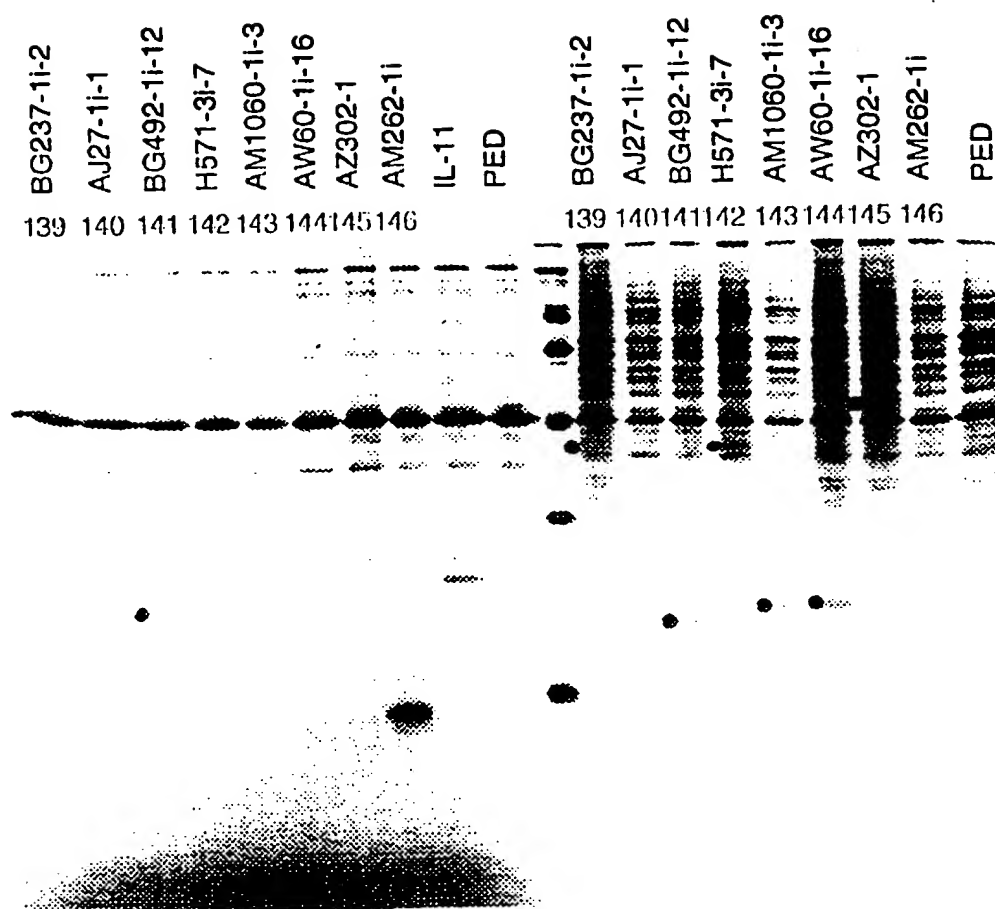


Fig. 2  
2/2

SUBSTITUTE SHEET (RULE 26)



**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/12, 15/19, C07K 14/52, 14/47,</b> <b>A61K 38/17, 38/19, C12N 5/10</b>	<b>A3</b>	<b>(11) International Publication Number: WO 97/46683</b> <b>(43) International Publication Date: 11 December 1997 (11.12.97)</b>
<b>(21) International Application Number:</b> PCT/US97/09878 <b>(22) International Filing Date:</b> 6 June 1997 (06.06.97)  <b>(30) Priority Data:</b> 08/659,224 7 June 1996 (07.06.96) <b>US</b>  <b>(71) Applicant:</b> GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US).  <b>(72) Inventors:</b> JACOBS, Kenneth; 151 Beaumont Avenue, Newton, MA 02160 (US). MCCOY, John, M.; 56 Howard Street, Reading, MA 01867 (US). LAVALLIE, Edward, R.; 90 Green Meadow Drive, Tewksbury, MA 01876 (US). RACIE, Lisa, A.; 124 School Street, Acton, MA 01720 (US). MERBERG, David; 2 Orchard Drive, Acton, MA 01720 (US). TREACY, Maurice; 93 Walcott Road, Chestnut Hill, MA 02167 (US). EVANS, Cheryl; Apartment #21, 35 Bellvista Road, Brookline, MA 02146 (US). BOWMAN, Michael; 50 Aldrich Road, Canton, MA 02021 (US). SPAULDING, Vikki; 11 Meadowbank Road, Billerica, MA 01821 (US).  <b>(74) Agent:</b> SPRUNGER, Suzanne, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).	<b>(81) Designated States:</b> AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 7 May 1998 (07.05.98)	
<b>(54) Title:</b> POLYNUCLEOTIDES ENCODING SECRETED PROTEINS  <b>(57) Abstract</b>  The invention provides 13 clones "AZ302-1" isolated from human colon; "AU139-2", "AU105-14", and "AJ147-1" from human adult testes; "AS268-1", "AS264-3", "AS301-2", "AS162-1" and "AS86-1" from human fetal brain; "D147-17" from human PBMC; "075-9" from human dendritic cells; "AM262-11" from human fetal kidney and clone "AR28-1" from human adult retina comprising polynucleotides encoding secreted proteins, using methods selective for cDNAs encoding secreted proteins.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Larvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

# INTERNATIONAL SEARCH REPORT

Inter national Application No  
PCT/US 97/09878

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/47 C12N15/19 C07K14/52 A61K38/19  
C12N5/10 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JACOBS K ET AL: "A NOVEL METHOD FOR ISOLATING EUKARYOTIC CDNA CLONES ENCODING SECRETED PROTEINS" JOURNAL OF CELLULAR BIOCHEMISTRY - SUPPLEMENT, vol. 21A, 10 March 1995, page 19 XP002027246 see abstract C1-207 ---	
X	J. NATHANS: "69C1 human retina cDNA Tsp509I-cleaved sublibrary Homo sapiens cDNA not directional" EMBL DATABASE ENTRY HSW22546, ACCESSION NUMBER W22546, 9 May 1996, XP002046017 see abstract --- -/--	1,13

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*Z\* document member of the same patent family

Date of the actual completion of the international search

11 November 1997

Date of mailing of the international search report

10.03.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

LE CORNEC N.D.R.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/09878

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CALLARD R E ET AL: "AIMS OF THE BOOK" CYTOKINE FACTSBOOK, 1994, CALLARD R E;GEARING A J H, pages 2/3, 31-38, 64/65, 75/76, 97/98, 148 - 151, 252/253, XP002039160 ---	
P,X	L. HILLIER ET AL: "The WashU-Merck EST project. zo29g02.r1 stratagene colon(#937204) Homo sapiens cDNA clone 588338 5'" EMBL DATABASE ENTRY HSAA51630 , ACCESSION NUMBER AA151630, 15 December 1996, XP002046018 see abstract ---	1,8-10, 13
A	ENG MONG LIM ET AL: "IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS DNA SEQUENCES ENCODING EXPORTED PROTEINS BY USING PHOA GENE FUSIONS" JOURNAL OF BACTERIOLOGY, vol. 177, no. 1, 1 January 1995, pages 59-65, XP000560419 ---	
A	M. YOKOYAMA-KOBAYASHI ET AL: "A signal sequence detection system using secreted protease activity as an indicator" GENE., vol. 163, 1995, AMSTERDAM NL, pages 193-196, XP002046435 ---	
T	US 5 536 637 A (JACOBS KENNETH) 16 July 1996 ---	
A	TASHIRO K ET AL: "SIGNAL SEQUENCE TRAP: A CLONING STRATEGY FOR SECRETED PROTEINS AND TYPE I MEMBRANE PROTEINS" SCIENCE, vol. 261, 30 July 1993, pages 600-603, XP000673204 ---	
T	K. A. JACOBS ET AL: "A genetic selection for isolating cDNAs encoding secreted proteins" GENE., vol. 198, 1 October 1997, AMSTERDAM NL, pages 289-296, XP002045919 -----	



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/09878

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 12 is directed to a method of treatment of the human/animal body (Rule 39.1 IV PCT), the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-13

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 97/09878

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## 1. Claims: 1-13

Polynucleotide sequence as in Seq. ID.2 from clone Az302-1 encoding secreted protein as in Seq. ID.3, fragments, compositions, potential therapeutic use and gene corresponding to the cDNA sequence ID.2, 1 or 4.

## 2. Claims: 14-16

Polynucleotide sequence as in Seq. ID.5 from clone AU139-2 encoding secreted protein as in Seq. ID.6, fragments, compositions and gene corresponding to the cDNA sequence ID.5 or 7.

## 3. Claims: 17-19

Polynucleotide sequence as in Seq. ID.8 from clone AU105-14 encoding secreted protein as in Seq. ID.9, fragments, compositions and gene corresponding to the cDNA sequence ID.8 or 10.

## 4. Claims: 20-22

Polynucleotide sequence as in Seq. ID.11 from clone AS268-1 encoding secreted protein as in Seq. ID.12, fragments, compositions and gene corresponding to the cDNA sequence ID.11 or 13.

## 5. Claims: 23-25

Polynucleotide sequence as in Seq. ID.21 from clone AJ147-1 encoding secreted protein as in Seq. ID.22, fragments, compositions and gene corresponding to the cDNA sequence ID.21 or 23.

## 6. Claims: 26-28

Polynucleotide sequence as in Seq. ID.24 from clone AM262-11 encoding secreted protein as in Seq. ID.25, fragments, compositions and gene corresponding to the cDNA sequence ID.24.

## 7. Claims: 29-31

Polynucleotide sequence as in Seq. ID.26 from clone AR28-1 encoding secreted protein as in Seq. ID.27, fragments, compositions and gene corresponding to the cDNA sequence ID.26 or 28.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 97/09878

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## 8. Claims: 32-34

Polynucleotide sequence as in Seq. ID.30 from clone AS162-1 encoding secreted protein as in Seq. ID.31, fragments, compositions and gene corresponding to the cDNA sequence ID.30, 29 or 32.

## 9. Claims: 35-37

Polynucleotide sequence as in Seq. ID.34 from clone AS264-3 encoding secreted protein as in Seq. ID.35, fragments, compositions and gene corresponding to the cDNA sequence ID.34, 33 or 36.

## 10. Claims: 38-40

Polynucleotide sequence as in Seq. ID.38 from clone AS301-2 encoding secreted protein as in Seq. ID.39, fragments, compositions and gene corresponding to the cDNA sequence ID.38, 37 or 40.

## 11. Claims: 41-43

Polynucleotide sequence as in Seq. ID.42 from clone AS86-1 encoding secreted protein as in Seq. ID.43, fragments, compositions and gene corresponding to the cDNA sequence ID.42, 41 or 44.

## INTERNATIONAL SEARCH REPORT

### Information on patent family members

Intern	1a) Application No
--------	--------------------

PCT/US 97/09878

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US. 5536637 A	16-07-96	US 5712116 A	27-01-98
-----			